

HUMAN UMBILICAL CORD-DERIVED HEMATOPOIETIC STEM CELLS IN CYTO- AND GENO-TOXICITY STUDIES: RESULTS WITH CADMIUM AND CHROMIUM COMPOUNDS

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The need of reliable, sensitive, specific and quick “in vitro” assays with direct extrapolability to humans has become compelling mostly for enlightening the cell mechanisms of oncogenic transformation and the toxic and carcinogenic potential of chemical compounds for human tissues. Human stem cells appear to be a reasonable alternative to non-human mammalian “in vitro” models or human genetically modified cell lines also in the light of the so-called “Stem cell theory of carcinogenesis”, for which a consistent body of evidences is emerging in recent years. Hematopoietic stem/early progenitor cells (HSC) were isolated, as expressing the CD34 marker, from human umbilical cord blood (UCB) by MACS technique (Magnetic Affinity Cell Sorting) and cultured in StemSpan serum-free expansion medium supplemented with StemSpan cytokine cocktail. HSC were exposed, following 10 day-expansion, to cadmium chloride (CdCl_2 ; 0.6, 3, 15, 75 and 375 μM) and sodium chromate (Na_2CrO_4 ; 12, 0.6, 3, 15 and 75 μM), and cell viability and proliferation were assessed by Trypan blue dye exclusion test. Na_2CrO_4 was more cytotoxic than CdCl_2 (after 2 day-exposure, an almost 100 % mortality with 15 μM Na_2CrO_4 and with 375 μM CdCl_2 was observed); cell death was mainly due to apoptosis only at the highest concentrations, as shown by morphological analysis following double staining with acridine orange/ethidium bromide. Cytokinesis-Block Micronucleus Assay (CBMN) and Single Cell Gel Electrophoresis (Comet Test) were performed to evaluate the genotoxic damage after 2 day-exposure of HSC to 0.6 and 3 μM Na_2CrO_4 and 3 and 15 μM CdCl_2 (at these concentrations, cell viability was not under 75%); a statistically significant increase in the frequency of binucleated cells with micronuclei was obtained only at the highest concentrations of both compounds; DNA damage/fragmentation, evaluated as “Tail Moment” and “Tail DNA(%)”, was induced by both concentrations of Na_2CrO_4 , but not by CdCl_2 . Ultrastructural analysis of human UCB CD34^+ HSC exposed to 3 μM Na_2CrO_4 or 15 μM CdCl_2 showed induction of autophagy, more pronounced with CdCl_2 in the presence of a slight increase in apoptotic cell death.

The obtained results, as compared with those obtained by us with cadmium (II) and chromium (VI) by using human normal (e.g. fibroblasts) or tumoral cell lines (e.g. melanoblasts), indicate that HSC are provided with higher resistance to both cytotoxic and genotoxic effects of these metals.